Wild barley shows a wider diversity in genes regulating heading date compared with cultivated barley

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Abstract

Heading date (HD) is an important agronomic trait that influences plant adaptability to varying environment and, ultimately, grain yield. In this study, two doubled haploid (DH) populations were used to identify new QTL for HD. One of DH population is originated from a cross between an Australian malting barley *cv.* Franklin and a wild barley accession TAM407227 and the other one is from the cross between a Syrian wild barley SYR01 and an Australian malting barley *cv.* Gairdner. Using three times of sowing (TOSs) differing in daylength and temperature, we investigated quantitative trait loci (QTL) controlling HD from both populations. Fourteen QTL were identified for HD from different populations and sowing dates. The expression of HD related genes varied with the TOS, suggesting a significant QTL × environment interaction. By comparing the positions of previously mapped HD genes and those of QTL detected in this population, we found that eleven of the fourteen QTL identified in this study were located at similar positions to those reported genes for HD. Among the three new potential QTL, one was located at 73.5 cM on chromosome 2H, explaining 19.2% and 4.6% HD of DH lines in spring and summer growing, respectively. The wild barley parent TAM407227 contributed the early maturity allele. HORVU2Hr1G088460 within the interval of QTL could be the candidate gene. The second new QTL was identified on chromosome 3H from a summer sowing trial and the third one on chromosome 4H affected HD of DH lines only under spring sowing condition. These new QTL identified will provide an alternative genetic resource for plant breeders developing barley varieties with improved HD adaptability to varying environments.

Key words: Barley; heading date; Quantitative trait locus

Introduction

As a period of transition from vegetative to reproductive growth, the HD is critically important to the propagation and survival of plant species, such that the success of pollination as well as seed development and dispersal requires flowering to occur at an optimum time. Like many agronomic traits, HD is controlled by quantitative trait loci (QTL) and is influenced by environment factors (Ellis et al. 1988; Hay and Kirby 1991). Fine-tuning the genetic basis of the HD is of a vital importance due to its key role in plant adaption to varying environments and in influencing the number of fertile florets and consequently grain yield (Fischer 2007). Regulation of HD in crops is also important considering the increasingly frequent occurrence of extreme climate events, especially temperature (Pachauri et al. 2014) and drought stress. By altering HD, plants avoid or escape water and temperature stresses. For example, as frost sensitivity increases after floral initiation (Fowler et al. 2001), delayed time of heading may assist in avoiding frost damage (Tsuda and Takami 1991). At the same time, earlier maturity is needed to avoid late season heat and drought stress (Cattivelli et al. 2010).

Dissection of molecular mechanisms underlying barley (*Hordeum vulgare* L.) HD will facilitate the development of both highly adaptive and productive barley varieties. Molecular markers have facilitated the dissection of quantitative traits into individual quantitative trait loci which are characterised by the construction of high-density genome linkage maps. In barley, the HD is generally determined by vernalisation requirements (Sayed et al. 2012), photoperiods (Roberts et al. 1988) and earliness *per se* (*Eps*) genes (Gallagher et al. 1991; Sameri et al. 2006). Principal genes involved in vernalisation and photoperiod pathways are responsible for controlling flowering time (Cockram et al. 2007; Distelfeld et al. 2009).

Depending on the requirement of prolonged environmental low temperature, barley can be classified into winter and spring types. Vernalisation accelerates flowering by promoting florescence initiation. Three genes, *VRN-H1*, *VRN-H2* and *VRN-H3* (Distelfeld et al. 2009; Hayes et al. 1993; Sasani et al. 2009), located on chromosomes 5H, 4H and 7H, respectively, are the major genes that operate in an epistatic fashion to determine the variation of vernalisation sensitivity. The recessive *Vrn-H1* accelerates the transition from the vegetative to the reproductive phase in barley (Hemming et al. 2008) whereas *Vrn-H2* represses heading in barley plants that have not experienced vernalisation. A *Flowering Locus T*-like gene, *HvFT1*, was identified as the main barley *FT*-like gene involved in switching to flowering and was proposed as a candidate gene for *VRN-H3* (Faure et al. 2007). Expression of *VRN-H3* is activated only after prolonged exposure to low temperature in winter varieties (Hemming et al. 2008; Yan et al. 2006). Others include floral repressors comprising MADS-box transcription factors, i.e., *HvVrt2*, *HvBM1* and *HvBM10* (Trevaskis et al. 2006; Trevaskis et al. 2007). These genes integrate light and temperature regulation of flowering possibly by acting downstream of *Vrn-H1* and *HvFT1* (Campoli et al. 2012).

Barley is a long day (LD) plant. The response of barley flowering time to LD period exposure can be weak or strong, depending on the versions of photoperiod response gene carrying different LD or short day (SD) sensitivity (Nishida et al. 2013). The major determinant of the LD response in barley is the *Photoperiod-H1* (*Ppd-H1*) locus (Laurie et al. 1995), which was mapped to chromosome 2H (Decousset et al. 2000; Laurie et al. 1995) and was identified as a pseudo-response regulator for adaption to photoperiod in barley (Turner et al. 2005). Dominant alleles at *Ppd-H1* confer early flowering under LD but have no effect under short days (SD). In contrast, the recessive allele *ppd-H1* is less responsive to photoperiod, due to altered circadian expression of the photoperiod pathway gene *CONSTANS* and reduced expression of its downstream target, *FT*, a key regulator of flowering (Turner et al. 2005). This reduced responsiveness allows an extended period of vegetative growth for spring-sown plants to accumulate additional resource. The observed variation in long photoperiod responsiveness can be explained by a single nucleotide polymorphism (SNP) within the exon 6 of the *Ppd-H1* coding region, with wild barley containing the nonresponsive version (Jones et al. 2008). Another major locus, *Ppd-H2*, was mapped on chromosome 1H (Laurie et al. 1995) and a candidate gene, *HvFT3*, was proposed for this locus (Faure et al. 2007).

Reproductive development is also regulated by "Earliness *per se*" (*Eps*) or *early maturity* (*eam*) genes , which functions independent of environmental cues, though this definition has been challenged with the report of thermos-sensitive *eps* genes (Bullrich et al. 2002). *Eps* genes play important roles in fine-tuning HD and anthesis (Griffiths et al. 2009) in barley and provide a wide adaption of plants to various environments. *Eps* loci have been identified on all chromosomes of barley (Gallagher et al. 1991; Laurie et al. 1995). Recessive mutants at some loci, including *eam7*, *eam8*, *eam9*, *eam10*, have been identified to confer early flowering (Börner et al. 2002; Franckowiak et al. 1997), photoperiod insensitivity and strong earliness effect under short days (Gallagher et al. 1987).

Many major loci controlling HD have been demonstrated or inferred to play pleiotropic roles in plant growth and development (Arifuzzaman et al. 2016; Li et al. 1995; Wang et al. 2010; Yan et al. 2011). *eps* genes also influence spike length and spikelet number (Ibrahim et al. 2018). The discovery of more HD genes will enable breeders to choose more suitable candidates in their breeding programs.

Wild barley (*Hordeum vulgare ssp. Spontaneum*) is the progenitor of cultivated barley (*Hordeum vulgare L.*). The long history of domestication has imposed selection pressure on the evolvement of many agronomic traits, leading to limited allele resources in cultivated barley (Ellis et al. 2000; Nevo and Chen 2010). Identification and utilisation of novel HD related genes originating from wild barley will provide breeders with more options to further improve the genetic basis of varieties for different purposes. Compared with cultivated barley, wild barley grows in a wider range of conditions, varying in temperature, day length and water availability (Ellis et al. 2000; Dai et al. 2012). Under harsh environments, early heading is an important mechanism to escape in cereals. Analysis of simple

sequence repeat (SSR) in wild barley has identified novel genomic regions involved in flowering time control, which had no association with photoperiodic and vernalisation response genes known to control flowering in cultivated barley (Ivandic et al. 2002). In the current study, we used two doubled haploid (DH) populations, both between a wild barley and cultivated barley. All DH lines and their parents were sown in three TOSs (winter, spring and summer) with varying environments (temperature and photoperiod), which enabled the understanding of the function of each HD QTL (i.e., expression activated/ deactivated or promoted/ suppressed) and their relative importance of deciding heading under specific environments. The overall aims of this study were to investigate the diversity in genes regulating HD between wild and cultivated barley and to identify new alternative QTL/genes for HD which could be delivered to breeders.

Materials and Methods

Plant material

Two DH populations were used in this study. The first population consisted of 163 lines originated from a DH population derived from the cross between TAM407227 (*Hordeum spontaneum*) and Franklin (*Hordeum vulgare* L). The second population consisted of 169 lines developed from a DH population derived from the cross between SYR01 (*Hordeum spontaneum*) and Gairdner (*Hordeum vulgare* L). The winter-type parent TAM407227 was introduced from Australian Grains Genebank, SYR01 is a Syrian wild barley introduced from China and both spring growth habit parent, Franklin and Gairdner are Australian malting barley varieties.

Field trial and HD measurement

To provide assessment of HD under contrasting environments, three trials were sown in winter, spring and summer, respectively, at Mt Pleasant Laboratory in Launceston, Tasmania, Australia (147°08′E, 41°28′S), which has a higher latitude than other barley growing regions in southern hemisphere. The first population were sown on 13 July, 12 September and 9 December 2016 for winter, spring and summer, respectively. The second population were sown on 17 May, 15 October and 15 November 2017 for winter, spring and summer, respectively. Forty seeds of each DH lines were sown in a 1.2 m row with a row spacing of 0.4 m. There were three replicates for each sowing time. For both trials, supplementary irrigation using sprinkler was provided in spring and summer, lasting one hour at middle of the day on a daily basis. Fungicide was applied every two weeks after seed germination.

HD was recorded as the days from sowing to the appearance of spikelets (Zadoks stages 51, Z51), different from flowering time (Alqudah and Schnurbusch 2017). The average data across three replicates of each sowing dates was used for QTL mapping.

Map construction

DH lines and their parental varieties of both populations were genotyped with DArTSeq (http://www.diversityarrays.com/dart-application-dartseq). A high-density map has been developed previously (Zhang et al. 2017). For the SYR01 x Gairdner cross, due to the large number of markers (~30,000 SNP and DArTSeq markers), those with the same positions or with greater distortion and/or greater proportion of missing data were removed from the map construction. A total of 3363 markers were used to construct the map using JoinMap 4.0 (Van Ooijen 2006). The marker positions were then aligned with the consensus map (Mascher et al., 2017).

QTL analysis

Data of days to heading for the two DH populations in each TOS was subjected to QTL analysis. Winter, spring and summer sowing were indicated as W, SP and SU respectively. Since winter type lines from the TAM407227 x Franklin population did not show heading in spring and summer sowing, these lines were excluded from QTL analysis and the analysis, which was indicated as SP-W and SU-W. For summer sowing, the DH lines were scored for winter (1) and spring habit (0), which were used to identify vernalisation related QTL. This trait was named SU+W.

The dataset was first analysed by interval mapping (IM). A screening threshold of logarithm of the odds (LOD) value of 3.0 was used to determine the presence of a QTL. The marker with the highest LOD value at each putative QTL identified using interval mapping was selected as a cofactor and were used as genetic background control in the approximate multiple QTL model (MQM) analysis of MapQTL6.0 (Van Ooijen and Kyazma 2009). The results were later subjected to restricted MQM (rMQM) mapping which does not use markers close to the QTL as cofactors. Two markers which were located left and right of the peak marker with LOD value attenuation of two were selected to define the intervals of each QTL (Van Ooijen and Kyazma 2011). The percentage of variance explained by each QTL (R^2) was obtained from rMQM mapping. The linkage groups and detected QTL with associated markers were incorporated into consensus linkage map using MapChart 2.2 (Voorrips (2002).

Functional annotation of putative genes linked to HD related markers

For the ease of comparison with genes identified in previous studies, the genetic position of QTL in this study were all converted into Morex consensus map location using the peak marker sequence to blast at IPK barley blast server (http://webblast.ipk-gatersleben.de/barley_ibsc/). The blast hit with the highest match was selected. The physical map of high confidence genes and functional description for identified novel QTLs were also generated. The generated barley high confidence genes with the highest score and lowest E value were selected as the most likely genes. Annotated functions in barley were downloaded from http://webblast.ipk-gatersleben.de/barley_ibsc/downloads/.

Naming of QTL

The names of QTL follow the rule of QTL(Q) for HD – population (TF or SG) – chromosome number – TOS (W etc.) – number of QTL on this chromosome if more than one QTL on a same chromosome. For example, *Qhd-tf-2Hsp1* means the first QTL for HD on chromosome 2H detected from spring sowing: w = winter sowing; sp = spring sowing; sp-w = the HD for winter type were deleted from spring sowing for QTL analysis; su = summer sowing; su-w: the HDs for winter type were deleted from summer sowing for QTL analysis; su+w: winter type lines was assigned as 1 and for spring type line as 0.

Statistical analysis

Normality test for heading date of each individual sowing was subjected to normality test using SPSS 19.0.0. The analysis of variance (ANOVA) was used to test the effect of genotype, environment and their interaction on heading date. ANOVA test was preformed using SAS 9.1.3. A threshold of P value ($P < 0.05$) was used to determine statistically significant difference.

Results

Phenotypic variation in HD for DH population

The weather condition (daily maximum and minimum temperature, daily rainfall) during the trial periods is shown in supplementary material (Online Resource 1). The frequency distribution of days-to-heading of the TAM407227 x Franklin population is shown in Fig. 1. The number of days required to heading of the DH lines varied with TOS and significant skewness (skewed towards earliness) was found in both spring and summer sown trials (Online Resource 2), which is not surprising as some of winter types gradually moved to reproductive stage due to lowering temperature towards winter time. Except for winter-type lines that showed no spiking in the summer trial, the length of time to heading ranges between 106-133 d in winter, 80-171 d in spring and 28-83 d in summer, respectively. Comparing with winter sowing, the longer period of daylength and higher temperature in spring largely accelerated initiation of heading in spring-type lines. During summer trials, days to heading were further reduced. There are approximately 120 lines which required less than 50 days to reach ear emergence whereas 14 lines did not show ear emergence throughout whole experiment period, which were regarded as winter barley. The wild barley parent TAM407227 was slightly later than Franklin under winter sowing but was earlier than Franklin under spring sowing. TAM407227 remained in the vegetative stage and did not turn reproductive under summer sowing.

For the SYR01 x Gairdner population, SYR01 was earlier than Gairdner in winter sowing but later than Gairdner in both spring and summer sowing (Fig. 2). DH lines showed a wide range of HD from the three TOSs. HD of the DH lines ranged from 140 to 174 DAS (days after sowing) in winter sowing, 43 to 100 DAS in spring sowing and 45 to 98 DAS in summer sowing trials with summer trial showing the shortest average time from sowing to heading although there were no substantial differences in HD

between lines sown in spring and summer. Table 1 shows that TOS, genotype and interaction between TOS and genotype all contributed significantly to HD ($P<0.0001$).

Mapping of HD QTLs

Fourteen QTL were identified from two DH populations. These QTL were located on all chromosomes (Table 2 & 3; Fig. 3). Among them, seven QTL were identified from both W and SP, four QTL from both SP-W and SU-W, and three QTL for SU+W (or SU). The identification of different QTL under varying environments suggested different genes were involved in HD regulation under different environments. QTL on chromosome 2H, 5H and 7H were repetitively detected in different populations and TOSs with minor shift of genetic distance of associated markers (Fig. 4). The details of detected QTLs are shown in Table 2 & 3, along with closest markers and confidence intervals. The QTL detected under all environments were confirmed by QTL analysis using phenotypic data per individual replication (Online Resource 3).

Winter sowing trial (W)

For the TAM407227 x Franklin population, the winter trial (W) identified most HD related QTL, distributed on all chromosomes except for 6H. These QTL explained 87.1% of phenotypic variation and were denoted as *Qhd-tf-1Hw, Qhd-tf-2Hw1, Qhd-tf-2Hw2, Qhd-tf-3Hw, Qhd-tf-4Hw, Qhd-tf-5Hw, Qhd-tf-7Hw*, respectively. Among them, the major effect QTL, *Qhd-tf-7Hw* was on chromosome 7H, accounting for 22% of phenotypic variation. The closest marker for this QTL was positioned at 24.5 cM on 7H. Another two major QTL were both found on 2H (*Qhd-tf-2Hw1* and *Qhd-tf-2Hw2*) and accounted for 19.2% and 18.7% of the phenotypic variation, respectively. TAM407227 contributed the earliness for early alleles for *Qhd-tf-2Hw1* (3.11 d earlier) and *Qhd-tf-7Hw* (3.05 d earlier) whereas Franklin had the early heading allele (2.97 d earlier than the late heading allele) for *Qhd-tf-2Hw2*. Comparing with these major QTL, the remaining four QTL contributed relatively less but significant effects. They are located at 132.4 cM of 1H (*Qhd-tf-1Hw*), 109.8 cM of 3H (*Qhd-tf-3Hw*), 51.4 cM of 4H (*Qhd-tf-4Hw*) and 122.6 cM of 5H (*Qhd-tf-5Hw*).

Only two major QTL were identified from the SYR01 x Gairdner population. These QTL accounted for nearly 48% of the phenotypic variation. The QTL on 2H, *Qhd-sg-2Hw*, is at a similar position to *Qhd-tf-2Hw1*. The other QTL on 3H, *Qhd-sg-3Hw*, is at a similar position to *Qhd-tf-3Hw*.

Spring sowing trial (SP, SP-W)

Only three QTL were detected in the TAM407227 x Franklin population, i.e. *Qhd-tf-2Hsp*, *Qhd-tf-4Hsp* and *Qhd-tf-5Hsp*. *Qhd-tf-2Hsp* and *Qhd-tf-5Hsp* are at similar chromosomal positions to *Qhd-tf-2Hw1* and *Qhd-tf-5Hw* identified in W, respectively. The closest marker for *Qhd-tf-2Hsp* is 3261247S2 (43.7 cM). This QTL had a confidence interval of 33.50~58.85 cM, which overlaps with *Qhd-tf-2Hw1* identified in W. Comparing the relative effect of each QTL to HD in W and S, *Qhd-tf-5Hsp* emerged

as the dominant QTL in SP, explaining 26.7% of the total variation. Another QTL, *Qhd-tf-4Hsp* was detected in SP which is located at a different position from the QTL on 4H detected in W. The nearest marker is 3265749D (115.1 cM) with the interval of 111.97~115.10 cM.

When winter-type lines were excluded for QTL analysis to eliminate the variance caused by vernalisation genes (SP-W), four QTL were detected. They are *Qhd-tf-2Hsp-w1*, *Qhd-tf-2Hsp-w2*, *Qhd-tf-4Hsp-w* and *Qhd-tf-7Hsp-w*, with genetic positions of 19.9 cM and 73.5 cM of 2H, 81.6 cM of 4H and 38.8 cM of 7H, respectively. *Qhd-tf-2Hsp-w1* is close to *Qhd-tf-2Hw2* identified in W and made the largest contribution to total phenotypic variation (43.2%). *Qhd-tf-2Hsp-w2* had a confidence interval from 67.28-74.15 cM, different from those other QTL on 2H. *Qhd-tf-7Hsp-w* was detected with an interval of 29.96~42.00 cM, similar to *Qhd-tf-7Hw* (23.79~32.79 cM) identified in W. The QTL on 4H (*Qhd-tf-4Hsp-w*) was some distance away from the one identified in W (*Qhd-tf-4Hw*).

Six QTL were identified in the SRY01 x Gairdner population, determining over 90% of phenotypic variation. The major QTL on 2H, *Qhd-sg-2Hsp*, determined 47% of the phenotypic variation. Other QTL were located on chromosomes 4H (*Qhd-sg-4Hsp*), 5H (*Qhd-sg-5Hsp1* and *Qhd-sg-5Hsp2*), 6H (*Qhd-sg-6Hsp*), 7H (*Qhd-sg-7Hsp*), determining 5% – 14% of phenotypic variation, respectively.

Summer trial (SU, SU-W, SU+W)

In the TAM407227 x Franklin population, four QTL were detected, with the two QTL on chromosome 2H (*Qhd-tf-2Hsu-w1*, *Qhd-tf-2Hsu-w2*) sharing the same marker with QTL identified in SP-W (*Qhd-tf-2Hsp-w1* and *Qhd-tf-2Hsp-w2*). The confidence interval of *Qhd-tf-7Hsu-w* overlaps both *Qhd-tf-7Hsp-w* and *Qhd-tf-7Hw*. Again, the highest R^2 was observed in *Qhd-tf-2Hsu-w1* (*Qhd-tf-2Hsp-w1*) accounting for 55.3%, followed by *Qhd-tf-7Hsu-w* (19.5%). When these DH lines were grouped to winter type (1) and spring type (0) according to the summer trial (S+W), only two QTL were detected on 4H (*Qhd-tf-4Hsu+w*) and 5H (*Qhd-tf-5Hsu+w*), at the same positions to *Qhd-tf-4Hsp* and *Qhd-tf-5Hsp*, respectively.

Three QTL were identified from summer sowing trial in the SRY01 x Gairdner population, two on 3H (*Qhd-sg-3Hsu1* and *Qhd-sg-3Hsu2*) and one on 5H (*Qhd-sg-5Hsu*). The major one, *Qhd-sg-3Hsu1*, with the closest marker of 3255135S5, explained 40% of the phenotypic variation.

Discussion

HD is an important agronomic trait for barley and many other crops. Genetic basis of the HD in barley has been investigated in many studies, with numerous genes being detected over entire barley genome (Fig. 3, Online Resource 4). The detection of multiple HD related QTL indicates the complex regulatory networks and great number of genes controlling this trait. Several pathways promote heading and flowering of barley, including vernalisation, photoperiod and earliness *per se* and gibberellin pathways (Boss et al. 2004). Previous studies have identified a large number of QTL/genes for HD (Backes et al.

1995; Cuesta-Marcos et al. 2008; Kjaer et al. 1995). However, most of these efforts have been focused on the QTL expressed under a single environment. The current experiments were conducted at 41°28′ S where daily rate of change in day length is greater than other barley growing regions in southern hemisphere. Effect of rate of change in daylength on the period from emergence to flowering have been reported in soy bean (*Glycine max* Merrill) (Constable and Rose 1988). However, rate of change of photoperiod was reported to have no effect on the rate of plant development in barley (Kernich et al. 1995). In this study, the approach of different sowing time enabled the identification of QTL potentially operating in different regulatory pathways. We have also used crosses between wild barley accessions and cultivated barleys, which ended up with a much greater number of QTL being identified.

Most of the QTL were located at similar positions to the known genes/QTL for HD

Two vernalisation sensitive QTL (*Qhd-tf-4Hsp/su+w, Qhd-sg-4Hsp/su, Qhd-sg-5Hsp2/su* and *Qhd-tf-5Hw/sp/su+w*) were identified. *Qhd-sg-5Hsp2/su* and *Qhd-tf-5Hw/sp/su+w* corresponded to *Vrn-H1*, which was established as a key vernalisation gene (Laurie et al. 1995). According to Cockram et al (2009), it is positioned at 125.76 cM. The effect of its collinear counterpart in Triticeae, *Vrn1*, has been well characterised (Fu et al. 2005; Yan et al. 2004). *Qhd-tf-4Hsp/su+w* and *Qhd-sg-4Hsp/su* represent another vernalisation gene *Vrn-H2* (Dubcovsky et al. 2005) at 101.36 cM on the long arm of chromosome 4H (Karsai et al. 2005; Szűcs et al. 2006). In contrast to *Vrn-H1* which was expressed under W, S and Sm+W, *Qhd-sg-4Hsp/su* and *Qhd-tf-4Hsp/su+w* was expressed only under longer daylength conditions (SP, SU and SU+W) and not detected under W, suggesting that the expression of *Vrn-H2* is highly sensitive to changes of environment cues or that *Vrn-H2* itself contributes very little to the timing of heading in this phase transition. In accordance with the former hypothesis, expression of *Vrn-H2* was reported to be highly dependent on daylength, with high level expression occurring under long daylength whereas *Vrn-H1* is photoperiod insensitive and is only affected by vernalisation and development (Trevaskis et al. 2006).

The QTL detected on chromosome 1H (*Qhd-tf-1Hw*) was at a similar position to *eam8* (Franckowiak 2002), a barley ortholog of the *Arabidopsis thaliana* circadian clock regulator *EARLY FLOWERING3* (*ELF3*) (Faure et al. 2012). The mutant for this gene, *mat.a-8,* confers early flowering associated with enhanced level of gibberellin. Using a set of recombinant inbred lines from a winter-type × spring-type cross, Sameri et al (2011) detected a QTL which corresponded to *eam8* on chromosome 1H and this QTL is photoperiod-sensitive.

Multiple QTL were detected on chromosome 2H. One of the QTL (*Qhd-tf-2Hw1/sp-w1*) showed a dominant role in determining HD in spring barley as it is the major contributor to HD in SP-W and SU-W, providing a potential to effectively regulate heading time of spring barley by changing the allelic form of this gene. The additive effect of TAM407227 allele was -3.05~-8.88, indicating the wild barley allele provides early heading. The QTL is co-located with *Ppd-H1* (Karsai et al. 1999; Turner et al.

2005). The importance of *Ppd-H1* revealed in our study is in accordance with previous findings that the pseudo-response regulator, *HvPRR37* (the candidate gene for *Ppd-H1*), is a major determinant of photoperiod sensitivity and flowering time (Turner et al. 2005). The other QTL on 2H (*Qhd-sg-2Hw*, *Qhd-tf-2Hw2* and *Qhd-tf-2Hsp*) was located at the similar position to an environment-independent earliness *per se* gene, *eps-2S* (*eam6*) near the centromere region of the 2H (Castro et al. 2017; Franckowiak and Konishi 2002; Laurie et al. 1995). The effect of *eps-2S* is more obvious under spring and autumn sowing conditions (Laurie et al. 1995). A QTL identified at similar position was found to expressed only in the Ppd-24 h treatment but not expressed under Ppd-10h, Phd-12h, Ppd-14h and Ppd-16h, suggesting that expression of this gene is sensitive to photoperiod (Sameri et al. 2011).

Qhd-tf-3Hw and *Qhd-sg-3Hw/su1* are located in the region of the semi-dwarf gene *sdw1* (*denso*) (Teplyakova et al. 2017), which is involved in the control of multiple agronomic traits, including reduction of plant height, yield components, lodging resistance and flowering time (Jia et al. 2011; Kuczyńska et al. 2014; Laurie et al. 1993) . This gene encodes gibberellic acid (GA)-20 oxidase (Jia et al. 2009). Both *sdw1* and *denso* alleles were reported to be associated with delayed heading (Powell et al. 1985; Thomas et al. 1991), potentially due to its reduced endogenous gas concentration (Jia et al. 2015).

Qhd-tf-4Hw is likely to be *eps-4L* (Yasuda 1978; Gallagher et al. 1991; Laurie et al. 1995). Also, in this region, several other QTL have been identified (Schmalenbach et al. 2009; von Korff et al. 2010; Ren et al. 2012). Except for QTL responding to the vernalisation gene *Vrn-H1* (*Qhd-tf-5Hw/sp/su+w and Qhd-sg-5Hsp2/su/su+w*), another QTL, *Qhd-tf-5Hsu-w* was only identified in SU-W. This QTL was expressed only under long days and acted more like *eam5*, which requires a minimum photoperiod to initiate flowering.

Within the interval of the QTL identified on 7H, there are two HD related genes, *eps-7S* (Laurie et al. 1995) and *Vrn-H3* (Takahashi and Yasuda 1971). The QTL on 7H is probably an *earliness per se* gene (*eps-7S*), as it was not detected under S+W. However, a previous study reported that *eps-7S* was only expressed under 24 h illumination, yet not expressed under 10~16 h photoperiod in the growth chamber, suggesting this locus is photoperiod sensitive (Sameri et al. 2011).

New potential QTL for the HD?

One of the QTL detected on 2H (*Qhd-tf-2Hsu-w2*) had a confidence interval of 67.28-74.15 cM, a region where no known HD related genes were reported in previous studies. Nearly 200 barley genes exist within the physical range corresponding to the genomic region from 67.28-74.15 cM (Online Resource 5). The closest marker is physically mapped to a high confidence gene HORVU2Hr1G088460, coding auxin-responsive *GH3* family protein. Other candidate genes in this region include auxin response factor 10 (*HORVU2Hr1G089660, HORVU2Hr1G089670*). *GH3* is one of the three primary auxin response genes and accumulates rapidly upon induction by auxin (Hagen and Guilfoyle 2002).

The *GH3* genes appear as gene family in *Arabidopsis* consisting of 20 members and have been shown to be involved in plant growth and development, photomorphogenesis, light- and auxin-signalling, and auxin homeostasis (Staswick et al. 2005).

No genes for the HD were reported in the region of *Qhd-tf-4Hsp-w* interval (78.68~85.84 cM), which include a total of 660 high confidence genes (Online Resource 6). Among those genes, *HORVU4Hr1G077360*, coding for ethylene-responsive transcription factor 1, is a candidate gene. *APETALA2* and *Ethylene Responsive Factor* (AP2/ERF) family, both belonging to AP2 superfamily, are involved in the regulation of plant metabolism, growth and development, and in responses to environmental stimuli. In *Arabidopsis*, *AP2-13* (*AP2*), *AP2-05* (*AINTEGUME NT, ANT*) and *AP2-09* (*ANT-LIKE1*) genes regulate floral growth and ovule development, respectively (Elliott et al. 1996; Klucher et al. 1996; Mizukami and Fischer 2000). Several AP2/ERF proteins are reported to regulate the development of shoot meristems (Licausi et al. 2013).

One of the QTL on 3H (*Qhd-sg-3Hsu*), which was expressed in summer sowing, was at 133.0 cM with a peak marker of 4596019D3. No genes were reported in this region which covered 692 high confidence genes (Online Resource 7). Among these genes, gibberellin (GA) 20 oxidase 2 (HORVU3Hr1G089980) and GA 20 oxidase 3 (HORVU3Hr1G090980) are likely the candidate genes. GA 20 oxidase is a key group of GA biosynthesis, the activity of which is increased by exposure to far red light (FR) -rich long day light (Gilmour et al. 1986). In *Arabidopsis thaliana*, *AtGA20ox2* is located on chromosome 4 whereas *AtGA20ox3* is located on chromosome 5 (Hisamatsu et al. 2005). Other candidates include cytochrome c-2 (HORVU3Hr1G090840), auxin efflux carrier family protein (HORVU3Hr1G094000), and auxin response factor 2 (HORVU3Hr1G096410, HORVU3Hr1G096510, HORVU3Hr1G097200).

In conclusion, the wild barley accession and cultivated barley examined here showed a wide diversity in genes regulating plant development, with a large number of QTL being identified from crosses between wild and cultivated barley. These QTL covered most of the reported genes for HD, including two major vernalisation genes. There were three potentially new QTL for HD, located on 2H, 3H and 4H, respectively. More studies are needed to confirm their roles and usefulness in breeding programs.

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Conflict of interest

The authors declare that they have no conflict of interest.

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561

562 **Figure captions**

563 **Fig. 1** Frequency distribution of phenotypes days to heading in the DH population of Tam407227 ×
564 Franklin based on means across replicates for three TOSs. Arrows indicate average days to heading
565 for parents.

566 **Fig. 2** Frequency distribution of phenotypes days to heading in the DH population of SYR01 ×
567 Gairdner based on means across replicates for three TOSs. Arrows indicate average days to heading
568 for parents.

569 **Fig. 3** LOD scans of restricted MQM mapping for days to heading in (a) 163 DH lines derived from
570 the TAM407227 and Franklin cross and (b) 169 DH lines derived from the SYR01 and Gairdner cross
571 in different TOSs. LOD curves for each TOS is stacked for comparison.

572 **Fig. 4** Genetic locations of QTL detected in this study and current known heading date (HD) genes
573 and QTLs adjusted to positions in Morex consensus map. QTL are shown as bars that indicate LOD-1
574 (solid box) and LOD-2 intervals (solid line), respectively. Red: known HD genes in barley; Other
575 colors indicate QTL detected in different TOSs. Blue: winter (W); dark green: spring (SP); light
576 green: spring-winter (SP-W); Pink: Summer, excluding winter type lines (SU-W for Tam407227 ×
577 Franklin population; SU for SYR01 × Gairdner population); Brown: SU+W for Tam407227 ×
578 Franklin population.

Table 1. ANOVA for days to heading of two DH populations sown in three TOSs.

DH population	Source of variance	Degree of freedom	Sum of Square	Mean Square	F Value	Pr > F
TAM407227 x Franklin	Genotype	162	142807	881	73	<.0001
	Time of sowing	2	1299956	649978	53939	<.0001
	Genotype × time of sowing	304	85191	280	23	<.0001
	Error	879	10592	12		
SYR01 x Gairdner	Genotype	170	79000	464	39	<.0001
	Time of sowing	2	2379210	1189605	99833	<.0001
	Genotype × time of sowing	243	57556	236	20	<.0001
	Error	826	9843	12		

Table 2. List of detected QTLs for heading time in the DH population from a cross between TAM407227 and Franklin sown in different TOSs

TOS[a]	Name[b]	Chr[c]	cM[d]	Marker[e]	Interval[f]	LOD[g]	R^2(%)[h]	Additive[i]	Putative Gene
W	*Qhd-tf-1Hw*	1H	132.4	3929594S1	127.36~132.40	5.96	5.7	-1.58	*eam8*
	Qhd-tf-2Hw1	2H	56.7	3810907D	53.26~59.42	13.77	19.2	-3.05	*eps-2S*
	Qhd-tf-2Hw2	2H	18.9	3259479S2	18.91~41.86	13.29	18.7	-2.97	*Ppd-H1*
	Qhd-tf-3Hw	3H	109.8	3265595S	104.32~116.40	9.27	9.3	-2.11	*sdw1*
	Qhd-tf-4Hw	4H	51.4	7931533D	50.99~62.54	8.20	8.1	1.9	*eps-4L*
	Qhd-tf-5Hw	5H	122.6	5255433S	122.57~128.19	4.39	4.1	1.54	*Vrn-H1*
	Qhd-tf-7Hw	7H	24.5	3255283S7	23.79~32.79	18.98	22.0	-3.11	*eps-7S*
SP	*Qhd-tf-2Hsp*	2H	43.7	3261247S2	33.50~58.85	5.98	11.0	-7.1	*eps-2S*
	Qhd-tf-4Hsp	4H	115.1	3265749D	111.97~115.10	6.79	12.6	7.31	*Vrn-H2*
	Qhd-tf-5Hsp	5H	125.8	7206897S	125.76~128.05	13.07	26.7	12.13	*Vrn-H1*
SP-W	*Qhd-tf-2Hsp-w1*	2H	19.9	3255173S2	18.90~26.20	21.85	43.2	-6.78	*Ppd-H1*
	Qhd-tf-2Hsp-w2	2H	73.5	7933859D	67.28~74.15	7.90	19.2	-4.65	
	Qhd-tf-4Hsp-w	4H	81.57	3264440S4	78.68~85.84	4.25	4.8	2.22	
	Qhd-tf-7Hsp-w	7H	38.8	3924463S	29.96~42.00	15.31	20.7	-4.62	*eps-7S*
SU-W	*Qhd-tf-2Hsu-w1*	2H	19.9	3255173S2	18.90~26.20	29.76	55.3	-7.16	*Ppd-H1*
	Qhd-tf-2Hsu-w2	2H	73.5	7933859D	67.28~74.15	4.46	4.6	-2.16	
	Qhd-tf-5Hsu-w	5H	139.2	4789985D	136.81~139.10	3.78	3.9	2.09	*HD6-5H?*
	Qhd-tf-7Hsu-w	7H	30.0	3398256S	27.83~34.21	15.58	19.5	-4.16	*eps-7S*
SU+W	*Qhd-tf-4Hsu+w*	4H	115.1	3273506D4	111.97~115.10	7.28	10.3	0.10	*Vrn-H2*
	Qhd-tf-5Hsu+w	5H	128.1	3271413S5	125.76~129.44	17.12	36.1	0.22	*Vrn-H1*

[a] Time of sowing (TOS) abbreviations: W: winter; SP: spring; SP-W: spring, excluding winter type lines for QTL analysis; SU-W: summer, excluding winter type lines for QTL analysis; SU+W: Value of 1 and 0 was assigned to winter type and spring type lines respectively for QTL analysis.
[b] Description of quantitative trait locus.
[c] Chromosome for detected QTL.
[d] Genetic position of peak marker position in Morex consensus map.
[e] Linked SNP or DArT marker.
[f] Confidence interval with LOD attenuation of 2.
[g] LOD value for peak marker.
[h] Effect (percentage of explanation) of QTL for total phenotypic variance.
[i] Additive effect, indicating the effect by the allele from TAM407227.

Table 3: List of detected QTLs for heading time in the DH population from a cross between SYR01 and Gairdner sown in different TOSs.

TOS[a]	Name[b]	Chr[c]	cM[d]	Marker[e]	Interval[f]	LOD[g]	R^2 (%)[h]	Additive[i]	Putative gene
W	*Qhd-sg-2Hw*	2H	43.7	11288299D	33.5-46.3	11.66	22.1	2.93	*eps-2S*
	Qhd-sg-3Hw1	3H	108.4	3254867S3	108-109.84	14.27	26.1	3.24	*sdw1*
SP	*Qhd-sg-2Hsp*	2H	18.9	3986258D2	18-19.9	17.36	47.1	8.29	*Ppd-H1*
	Qhd-sg-4Hsp	4H	112	3664225D4	110.1-112.2	3.14	6.5	-0.17	*Vrn-H2*
	Qhd-sg-5Hsp1	5H	44.17	3985340D5	41.81-45.51	3	5.9	-0.11	*HvCO3;HvTFL1;HvCFM13*
	Qhd-sg-5Hsp2	5H	125.8	3263359D5	125-129.8	6.29	13.9	-0.15	*Vrn-H1*
	Qhd-sg-6Hsp	6H	68.2	3269813D6	65.44-69.05	3.03	10.3	3.28	*HvCO14;HvCO2;HvCo11*
	Qhd-sg-7Hsp	7H	6.76	5240745D7	6.8-13.6	3.89	7.4	-0.14	*Eps-7S?*
SU	*Qhd-sg-3Hsu*	3H	133	4596019D3	132-133	3.63	7.0	3.24	
	Qhd-sg-5Hsu	5H	112.71	3987226D5	112.03-129.8	4.31	11.1	-0.13	*eam5; HvPhyC*

[a]Time of Sowing (TOS) abbreviations: W: winter; S: spring; Sm: summer.
[b] Description of quantitative trait locus.
[c] Chromosome for detected QTL.
[d] Genetic position of peak marker position in Morex consensus map.
[e] Linked SNP or DArT marker.
[f] Confidence interval with LOD attenuation of 2.
[g] LOD value for peak marker.
[h] Effect (percentage of explanation) of QTL for total phenotypic variance.
[i] Additive effect, indicating the effect by the allele from SYR01.

Supplementary material

Online Resource 1. Weather data (daily maximum and minimum temperature, rainfall) of Launceston during trials of different TOSs.

Online Resource 2. Normality statistical test (skewness and kurtosis) for heading date of two DH populations with different TOSs.

Online Resource 3. Confirmation of QTL mapping results using phenotypic data per individual replication for each trial.

Online Resource 4. Major HD related genes, primer/marker sequences and genetic locations on consensus map. Only genes with available reported primer/ marker are shown in the table.

Online Resource 5. 193 barley high confidence genes within the confidence interval of *Qhd-tf-2Hsp-w2*.

Online Resource 6. 660 barley high confidence genes within the confidence interval of *Qhd-tf-4Hsp-w*.

Online Resource 7. 692 barley high confidence genes within the confidence interval of *Qhd-sg-3Hsu.*